PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/35, 31/235, 31/165, 31/12, 31/075, 31/135, 31/24, C07D 311/04, C07C 49/105, 49/76

A1

(11) International Publication Number: **WO 99/22728**

(43) International Publication Date: 14 May 1999 (14.05.99)

(21) International Application Number: PCT/US98/23041

(22) International Filing Date: 30 October 1998 (30.10.98)

(30) Priority Data:
60/063,770 31 October 1997 (31.10.97) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: METHODS AND COMPOSITIONS FOR REGULATION OF 5-ALPHA REDUCTASE ACTIVITY

(57) Abstract

Compounds that inhibit 5α-reductase are provided. The compounds are used to treat prostate cancer, breast cancer, obesity, skin disorders and baldness.

METHODS AND COMPOSITIONS FOR REGULATION OF 5-ALPHA REDUCTASE ACTIVITY

Technical Field of The Invention

The present invention relates generally to compounds, compositions and methods regulating the action and function of androgens and other steroid hormones by modulating the activity of steroid-reductases, including isozymes of 5α-reductases. More specifically, the present invention relates to the use of these compounds to regulate processes or treat disorders that are modulated by androgens or other steroid hormones or are caused by abnormal actions of androgens or other steroid hormones in cells or organs of animals, humans, plants, or microorganisms. This invention relates to the use of natural and synthetic flavanoids, catechols, curcumin-related substances, quinones, catechins and fatty acids and their analogues or derivatives as 5α-reductase isozyme inhibitors and as therapeutic agents. These compounds can also be used in promoting or modulating desirable production of specific products for commercial purposes.

Background of the Invention

In some of the androgen-sensitive organs, such as the prostate and skin, testosterone (T) is converted to a more active metabolite 5α-dihydrotestosterone (DHT) by 5α-reductase (Anderson and Liao, 1968; Bruchovsky and Wilson, 1968). Other substrates of 5α-reductases are also converted to reduce products that may have specific properties. Inhibition of 5α-reductase represents a unique approach for developing therapeutic methods for androgen-dependent diseases, such as benign prostatic hyperplasia, breast and prostatic cancer, skin disorders, seborrhea, common baldness, hirsutism, and hidradenitis suppurative. Various compounds have been shown to inhibit 5α-reductase activity (Liang and Liao, 1992; Hirsch et al., 1993; Russell and Wilson, 1994; Liao and Hiipakka, 1995). Finasteride (Proscar), a 5α-reductase inhibitor, lowers the level of DHT in serum and the prostate, reduces prostate volume and increase urinary flow in some patients (Stoner E. Finasteride Study Group, 1992). Certain aliphatic unsaturated fatty acids, such as γ-linolenic acid (Liang and Liao, 1992) and catechin-3-gallates (Liao

males in the U.S. Male-patterned baldness can start as early as the teens in genetically susceptible males, and it has been estimated to be present in 30% of Caucasian males at age 30, 40% of Caucasian males at age 40, and 50% of Caucasian males at age 50. Acne is the most common skin disorder treated by physicians. In women, hirsutism is one of the hallmarks of excessive androgen. The ovaries and the adrenal are the major sources of androgen in women.

In men, the major androgen circulating in the blood is testosterone. About 98% of the testosterone in blood is bound to serum proteins (high affinity finding to sex-steroid binding globulin and low affinity binding to albumin), with only 1-2% in free form. The albumin-bound testosterone, the binding of which is readily reversible, and the free form are considered to be bioavailable, and account for about 50% of total testosterone. Testosterone enters target cells apparently by diffusion. In the prostate, seminal vesicles, skin, and some other target organs, it is converted by a NADPH-dependent 5α-reductase to a more active metabolite, 5α-DHT. 5α-DHT then binds an androgen receptor (AR) in target organs. The 5α-DHT-receptor complexes interact with specific portions of the genome to regulate gene activities (Liao et al., 1989). Testosterone appears to bind to the same AR, but it has a lower affinity than 5α-DHT. In tissues such as muscle and testes, where 5α-reductase activity is low, testosterone may be the more active androgen.

The difference between testosterone and 5α-DHT activity in different androgen-responsive tissues is further suggested by findings in patients with 5α-reductase deficiency. Males with 5α-reductase deficiency are born with female-like external genitalia. When they reach puberty, their plasma levels of testosterone are normal or slightly elevated. Their muscle growth accelerates, the penis enlarges, voice deepens, and libido toward females develops. However, their prostates remain non-palpable, they have reduced body hair, and they do not develop acne or baldness.

The findings in 5α-reductase deficient patients suggest that inhibitors of 5α-reductase would be useful for the treatment of prostatic cancer, BPH, acne, baldness, and female hirsutism. Clinical observations and animal experiments have indicated that spermatogenesis, maintenance of libido, sexual behavior, and

some cases, to decrease the weight of an androgen-dependent body organ, such as the prostate and other organs. The effectiveness of these compounds may be dependent also on their action on other mechanisms involved in angiogenesis, cell-cell interaction, and on their interaction with various components of organs and cells.

Compounds useful in the practice of the present invention include various isomers of saturated and unsaturated fatty acids, natural and synthetic analogues, and derivatives from which these fatty acids can be generated as well as the metabolites and oxidation products of these fatty acids. The use of these and other fatty acids and their derivatives is also contemplated. Also useful are catechin compounds, particularly, catechins that are structurally similar to epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). EGCG has an additional hydroxyl group on the epicatechin gallate molecule, which has been found to be surprisingly active in modulating several 5α-reductase mediated processes. EGCG derivatives having such an additional OH group on the altering ECG molecule were shown to be active in inducing body weight loss and particularly in reducing the size of androgen sensitive organs such as preputial glands, ventral prostate, dorsolateral prostate, coagulating glands, seminal vesicles, human prostate tumors, and breast tumors in nude mice.

By analogy with the fatty acid compounds, certain active catechin gallates may not enter target cells easily. However, esterification of hydroxyl groups on the inhibitor compounds should enhance the ability of these compounds to enter the target cells. Once inside the cells, esters would be readily hydrolyzed by esterase to alcohols that can inhibit 5α-reductases (Williams, 1985).

In more particular aspects of the invention, the inventors have discovered that certain catechins, particularly EGCG, can be administered to promote body weight loss that differentially affects overall body weight and prostate weight loss. In particular examples, it was shown that for a certain percentage of overall body weight loss, prostate weight loss was percentage-wise more than three times as much. The loss in body weight and the organ weight are likely due to EGCG interference of a common step in the pathway controlling body weight and the

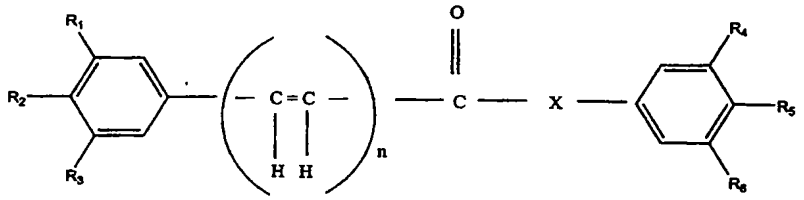
which the compounds are delivered to target sites. Such a delivery system may involve, among other methods, liposome techniques or immunological devices.

Natural or synthetic chemicals that can modulate the production or cellular action of receptors and macromolecules are useful in the treatment of abnormalities such as obesity, BPH, prostate cancer, skin diseases, baldness, breast tumors, and hirsutism, which are related to lipid synthesis, body weight, and/or androgen function.

Animal models can be used to demonstrate the effectiveness of compounds on a variety of cancers. For example, Shionogi tumor and other tumors can be studied in male rats. Human breast and prostate cancer cell growth can be studied in nude mice. Alternatively, rodent breast tumors induced by carcinogens and other cancers induced in transgenic mice or Dunning tumor in rats can be similarly analyzed for their chemotherapy by EGCG and related compounds.

The use of compounds disclosed in the present invention, or in natural therapeutically effective amounts of pharmaceutical compositions containing one or more of the compounds, in some cases in combination with other therapeutic agents and carriers, or in natural or synthetic products, is appropriate in the treatment of various disorders. These disorders include, but are not necessarily limited to, those conditions wherein excessive androgenic activities have been implicated, for example, male pattern baldness, female hirsutism, skin disorders, BPH, cancers of prostate, breast, skin and other organs.

The present invention is also directed to novel compounds. These compounds have the formula:



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where x is $-NHCH_2CH_2-$ or -CH=CH-;

R_1, R_2 and R_3 each may be -H, -OH or $-OCH_3$, provided that only one of R_1, R_2, and R_3 may be -H;

All of the compositions and methods disclosed and claimed herein can be made without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Brief Description Of The Drawings

In the drawings, which form a portion of the specification:

Fig. 1 shows the structure of flavanoid compounds of the present invention.

Fig. 2 shows the structure of catechol compounds of the present invention.

Fig. 3 shows the structure of curcumin and related compounds of the present invention.

Fig. 4. shows quinones of the present invention.

Fig. 5 shows epigallocatechin derivative compounds of the present invention.

Fig. 6 shows the generic formula of the epigallocatechin derivatives of the present invention;

Fig. 7 shows the generic formula of gallates useful in the present invention;

Fig. 8 shows the generic formula of curcumin derivatives useful in the present invention;

Fig. 9 shows the generic formula of quinones and catechols useful in the present invention.

Fig. 10 shows fatty acids of the present invention.

and washing with 10 ml of 20 mM-potassium phosphate, pH 7.0, containing 0.01% CHAPS to remove unbound [^{3}H]4-MA.

B. Assays Of The Enzymatic Activity Of Microsomal 5α-Reductase

The standard reaction mixture, in a final volume of 0.15 ml, contains microsomes, 1 μCi of [^{3}H]testosterone, 0.5-3.0 μM non-radioactive testosterone, 0.1mM-NADPH, 1mM-dithiothreitol and 50 mM-potassium phosphate, pH 7.0, with or without the indicated amount of a lipid or an inhibitor preparation. The reaction is started by the addition of microsomes and the incubation is carried out at 37° C. for 15 minutes. Steroids are extracted and separated by thin layer chromatography. Radioactive steroids are located by fluorography and the amount of radioactivity present determined by scintillation counting. The 5α-reductase activity was measured by analyzing the extent of the conversion of [^{3}H]testosterone to [^{3}H]5α-DHT.

C. Sources of 5α-Reductase Activity

Microsomes are prepared at 4° C. from a buffered 0.32 M-sucrose homogenate of human liver and from the livers of adult Sprague-Dawley female rats by differential centrifugation, and are used in the assay of 5α-reductase activity. In some experiments, microsomes are solubilized with 0.1% polyoxyethylene ether W-1, except for the substitution of polyoxyethylene ether W-1 for Lubrolx-WX.

Cells genetically engineered to express specific types of 5α-reductase isozymes can also be used as sources of 5α-reductase activity. Intact cells containing 5α-reductase, their microsomes, or nuclear preparations can also be used to screen 5α-reductase inhibitors.

II. Prostate And Breast Cancer

A compound of this invention can be used to treat breast or prostate cancer. The effectiveness of such compounds against prostate and breast cancer can be determined either on isolated cell lines derived from such cancer tissues or in animals demonstrating these forms of cancer. By way of example, human prostate cancer PC-3 cells are grown in culture medium. About one million cells are injected into male nude mice and the growth of tumors followed. Within two

maintained individually in a plastic cage on rodent chow (Purina) and water ad libitum on a 12 hour light/12 hour dark cycle.

One to two weeks after castration, the hair on the lower back of each animal is clipped with an electric hair clipper and then shaved weekly to expose the flank organs. The animals are divided into treatment groups. A treatment solution (5 μl) is applied topically to the right flank organ once a day using a Pipetteman and a polypropylene disposable tip. Unless specified, the left flank organ is not treated. The treatment solution contains either (a) ethanol alone (vehicle and control), or (b) a test compound. The flank organ was wiped with an alcohol pad to remove residual compound before each treatment. At the end of each experiment (17-25 days), the animals were sacrificed by either suffocation with CO_2 gas or with an intraperitoneal injection of an overdose of phenobarbital (64.8 mg/ml/animal). The flank organs, both the treated and untreated sides, are evaluated to determine the effect of these treatments on the growth of the pigmented macule and the sebaceous glands. The body weight of each animal is recorded before and after treatment.

Treatment Of Animals

Male hamsters, 4 weeks old, are castrated and are kept on a longer light period (16 hours light/8 hours dark cycle) to insure maximum stimulation of sexual characteristics (Luderschmidt et al., 1984). Flank organs, left and right, were treated topically with 5 μl ethanol containing 0.5 μg or 1 μg testosterone daily. Animals are divided into groups of 4-5 hamsters. The right flank organ is also treated daily with 5 μl solution containing vehicle (ethanol) alone or test compound (1 or 2 mg) for 18 days. The left flank organ of all animals receives the same volume of vehicle.

the lengths of the long axis and the short axis of the pigmented spot (pigmented macule) are measured using a caliper with digital display (Digimatic, Mitutoyo Corp., Japan). The product (long axis x short axis, mm^2) is used as an index of the surface area (Wuest and Lucky, 1989).

The flank organ treated with test compound becomes elevated and palpable. The length of the long axis and short axis of the elevated mass are measured with a caliper. The product of the long axis x short axis (mm^2) was used as an index of the

Determination Of Forehead Sebum Production

A male volunteer is used to test and analyze sebum production from the forehead region. The forehead is washed thoroughly with soap twice and then cleaned with 70% isopropyl alcohol twice. Sebum production is measured 30 to 60 minutes later with a sebum meter tape probe (7 mm x 8 mm) covering 56 mm^2 area in each measurement. Ten measurements are made within the 4 cm square area ($16cm^2$) located at the middle of the left or right side forehead between the eyebrow and the hair line.

The sebum meter detects the difference in the transparency of the tape before and after the tape was placed on the forehead for 30 seconds and expresses the difference in an arbitrary number (S-value) between 0 to 300 (or higher). S-values of sebum accumulated on the foreheads of men are usually 200 to 300. Skin surface on hands usually shows a very low number (5 to 20). The S-value for forehead immediately after washing is less than 5. For men, the S-value gradually increases to about 50 within 30 minutes after washing and reaches 100 to 200 in 45 minutes to 55 minutes.

To determine the rate of sebum production, the left and the right forehead areas are measured alternatively and each time at the comparable areas on the two sides. Ten measurements on each side (i.e., 20 measurements for two sides) take about 15-20 minutes and the sebum-values likely range between 30 to 200. The S-values can differ considerably at different areas of the forehead and could be influenced by environmental, including weather, diet, and physiological, conditions. However, the ratio of the total S-value (the sum of 10 measurements) for the left and the total S-value for the right forehead is constant. Therefore, compounds applied to the left forehead that reduce the L/R ratio to lower than 1.1 are considered as topically active agents for suppression of sebum production.

VII. Pharmaceutical Compositions

Aqueous compositions of the present invention comprise an effective amount of the 5α-reductase inhibitory agent dissolved or dispersed in a pharmaceutically acceptable aqueous medium. The phrase "pharmaceutically

material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic. In addition, the active compounds may be incorporated into sustained-release preparations and formulations.

The active compounds may also be administered parenterally, intravenously, or intraperitoneally. Solutions of the active compounds as a free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquified polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such a lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for

Sciences", 15th Edition). Variation of the dosage of the compositions disclosed herein, will necessarily depend upon the particular subject, and the nature of the condition(s) being treated.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermic or intravenous fluid or injected at the proposed site of infusion, (see, for example, "Remington's Pharmaceutical Sciences", 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

EXAMPLE

Assays For Candidate Substances

Expression Of Human 5α-Reductases. For the preparation of rat 1A cells expressing different types of human 5α-reductases, cDNAs for the human type 1 and 2 5α-reductases were isolated from human prostate λgt11 and PC-3 cell λZAP II cDNA libraries using the published sequence of the 5α-reductases, PCR and standard library screening techniques. The type 1 and 2 cDNAs were subcloned into the retroviral expression vector pMV7 and high titer stocks of virus containing the type 1 and 2 cDNAs were generated using the packaging cells BOSC 23 293. Rat 1A cells were infected with virus and cells containing integrated retrovirus were selected for G418 resistance (Brown and Scott, 1987).

Assay Of 5α-Reductase. Microsomes were prepared from rat 1A cells expressing specific types of human 5α-reductase. The enzymic assay was based on

11. Epigallocatechin	>100	15	>100	3
12. Epicatechin	>100	14	>100	4
13. Morin	>100	6	>100	33
14. Alpha-napthoflavone	>100	6	>100	(-13)
15. Taxifolin	>100	5	>100	5
16. Rutin	>100	4	>100	0
17. Daidzein	>100	3	29	69
18. Beta-napthoflavone	>100	3	>100	4
19. Chrysin	>100	2	>100	1

The tea catechins, ECG and EGCG, had the highest activity of the tested flavanoids and were better inhibitors of the type 1 (HRED1) than the type 2 (HRED2) isoenzyme of 5α-reductase. The tea catechins epicatechin (EC) and epigallocatechin (EGC) had little activity. Four flavanoids, myricetin, quercitin, baicalein and fisetin had significant (IC50<100 μM) activity and were more active against the type 1 than the type 2 isoenzyme. Biochanin A, kaempferol, genistein, and diadzein were effective inhibitors of the type 2 but not type 1 isoenzyme. Comparison of the activities of chrysin, kaempferol, morin, myricetin, and quercitin indicate the importance of B-ring hydroxyl groups, especially in a catechol or pyrogallol configuration, and perhaps the importance of the hydroxyl at position 3 for activity against the type 1 isozyme. Rutin, the 3-rutinose glycoside of quercitin was ineffective against either isoenzyme (IC50>100 μM). The inactivity of rutin compared to quercitin is either due to the presence of the oligosaccharide rutinose (perhaps due to steric hindrance) or modification of the hydroxyl at position 3. Taxifolin, a flavanone, was ineffective against either isozyme (IC50>100 μM). The weak activity of taxifolin is most likely due to the absence of the 2,3-unsaturated bond when its activity is compared to the structurally related quercitin. When tested for inhibitory activity in whole cells, most flavanoids showed little or no activity against the type 1 isoenzyme, perhaps indicating limited penetration of these polyhydroxy compounds across the cell membrane. In contrast to the results with the type 1 enzyme, four flavanoids, biochanin A, diadzein, kaempferol and

18. HZIV 275	>100	10	>100	6
19. Exculetin	>100	7	>100	13
20. Ellagic Acid	>100	7	>100	9
21. Catechol	>100	5	>100	0
22. Methylgallate	>100	5	>100	3
23. Fraxetin	>100	2	>100	8
24. Propylgallate	>100	0	>100	0

Thirteen of the 24 compounds listed had IC50's below 100 μM. All were more active against the type 1 than type 2 isoenzyme. Six of these compounds, anthrarobin, dodecyl gallate, gossypol, octyl gallate, caffeic acid phenethyl ester and nordihydroguaiaretic acid were active in whole cell assays (Table 7, below). Anthrarobin was much more effective against the type 1 than type 2 isoenzyme; whereas, the other five inhibitors were equally effective inhibitors of both isoenzymes. The synthetic compound HZIV 82 showed little activity in the cell-free assay, but was very active in the whole cell assay with specificity for the type 1 isoenzyme.

3. CURCUMIN AND RELATED COMPOUNDS

Curcumin was a very effective inhibitor of either the type 1 or type 2 isoenzyme (Table 3, Fig. 3).

TABLE 3

Cell Free Assay Isoenzyme / Compound	HRED1 IC50(uM)	HRED1 % Inhibition @ 100 uM	HRED2 IC50(uM)	HRED2 % Inhibition @ 100 uM
1. Curcumin	3	95	5	87
2. Tetrahydrocurcumin	80	56	29	73
3. Demethoxy-tetrahydrocurcumin	>100	23	>100	42
4. 4-hydroxy-3-methoxy-cinnamaldehyde	>100	10	>100	(-60)
5. Coniferol	>100	10	100	49

8. Anthrarufin	40	67	>100	13
9. Anthrarufin	40	67	>100	13
10. Lapachol	>100	30	>100	9
11. Anthraflavic Acid	>100	27	>100	22
12. Quinizarin	>100	26	>100	7
13. T-butylhydroxyquinone	>100	19	>100	4
14. Anthraquinone	>100	6	>100	9

The naturally occurring anthraquinone, alizarin, was a very effective inhibitor of the type 1 but not type 2 isozymes. Alizarin Red S, which is a water soluble sulfate derivative of alizarin had little activity (IC50s>100 μM) against either isoenzyme. The charged sulfate group may prevent interaction with membrane bound 5α-reductase. Purpurin, which has an additional hydroxyl compared to alizarin, had inhibitory activity similar to alizarin. Anthraflavic acid, anthrarufin and quinizarin, which are structural isomers of alizarin without adjacent hydroxyl groups, had much less activity, emphasizing the importance of the catechol moiety for potent inhibitory activity of this class of anthroquinones. Anthraquinone was not an effective inhibitor (IC50>100 μM). Menadione, coenzyme Q, and 2,6-dichloroindophenol were potent cell-free inhibitors of boh isoenzymes. The compounds participate in quinone reductase reactions and may deplete NADPH causing the observed inhibition. In the whole cell assay, alizarin was a very effective inhibitor of the type 1 isoenzyme and menadione had moderate activity.

5. EPIGALLOCATECHIN DERIVATIVES

The high inhibitory activity of EGCG in a cell-free assay but low in the whole cell assay led us to design and synthesize a series of derivatives of EGC to enhance activity in the whole cell assay (Table 5, Fig. 5).

structural changes are summarized in Table 5. The most significant structural change leading to activity in the whole cell assay was introduction of fatty acid ester in place of the gallic acid group of EGCG. In particular, fatty acids with some degree of unsaturation had good inhibitory activity against both isoenzymes of 5α-reductase in the whole cell assay. The most potent of these derivatives was one with γ-linolenic acid esterified to the 3-hydroxyl of EGC. Certain fatty acids with a single unsaturated bond were also active. For example, HZIV 160, the myristoleic ester of EGC was effective in both assay systems. Fatty acids with less unsaturation are less susceptible to oxidation and so may be more suitable modifying agents.

TABLE 6

Cell Free Assay Isoenzyme / Compound	HRED1 IC50(uM)	HRED1 % Inhibition @ 100 uM	HRED2 IC50(uM)	HRED2 % Inhibition @ 100 uM
1. Gamma-Linolenic Acid C18:3 CIS 6.9,12	5	99	11	89
2. Crocetin	7	70@30	>100	20@30
3. Alpha-Linolenic Acid C18:3 CIS 9,12,15	8	99	9	84
4. Linoleic Acid C18:2 CIS 9,12	9	99	19	85
5. Oleic Acid C18:1 CIS 9	10	99	42	86
6. Conjugated Octadecadienonic Acid	10	99	30	81
7. 5,8,11,14-Eocpsatetraynoic Acid	15	97	3	81
8. Stearic Acid C18:0	27	71	>100	35

25. Fisetin	>100	42	>100	27
26. EGCG	>100	11	>100	5
27. Myricetin	>100	11	>100	11
28. Purpurin	>100	47	>100	7
29. Quercetin	>100	15	>100	29
30. Alizarin Red S	>100	28	>100	1
31. Genistein	>100	22	20	89
32. HZIV 123	>100	48	>100	8
33. HZIV 107	>100	23	>100	2
34. Catechol	>100	9	>100	3
35. Daidzein	>100	9	58	87
36. Pyrogallol	>100	7	>100	15
37. EC	>100	0	>100	1
38. EGC	>100	15	>100	1
39. ECG	>100	0	>100	0
40. EGCG	>100	6	>100	0
41. HZIV 90	>100	34	>100	14
42. HZIV 63	>100	12	>100	7
43. HZIV 68	>100	40	>100	34
44. HZIV 144	>100	12	>100	7
45. HZIV 81-3	>100	28	19	80
46. HZIV 145	>100	8	>100	9
47. Methyl Gallate	>100	0	>100	0
48. Propyl Gallate	>100	5	>100	0
49. Isopropyl Gallate	>100	0	>100	0
50. Gallic Acid	>100	13	>100	0
51. Pyrogallol	>100	5	>100	6
52. HZIV 169	>100	10	>100	0
53.Gamma-Linolenic	22	91	20	86
54. Etya	22	67	2	86

inorganic acid, such as hydrochloric acid, sulfuric acid, or phosphoric acid, or an organic acid, such as citric acid or acetic acid.

The references listed below and cited in the disclosure are:

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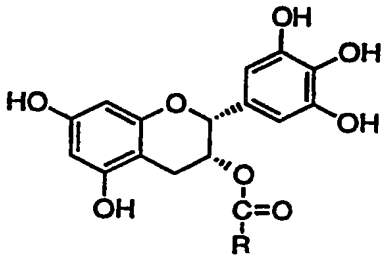
R_4, R_5 and R_6 each may be -H, -OH, -OCH_3 or -$N(CH_3)_2$, provided that only one of R_4, R_5 and R_6 may be -H; and

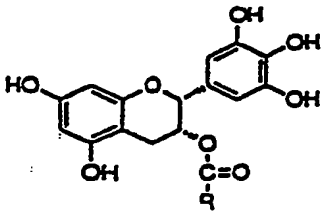
n is 0 or 1.

9. A compound of the formula:



where R is a chain with 2 to 20 atoms from the group consisting of carbon, oxygen, sulfur, and nitrogen, without or with one to four double bonds and additional hydrogen.

10. A compound of the formula:



where R is

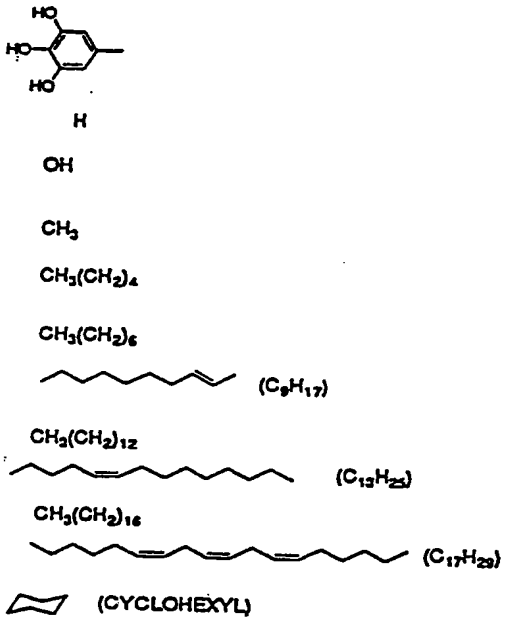
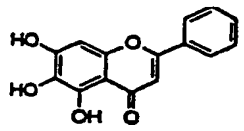


FIGURE 1 - FLAVANOIDS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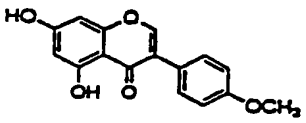
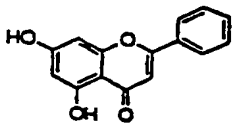
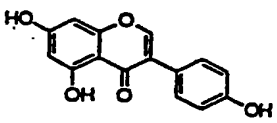

BIOCHANIN A


CHRYSIN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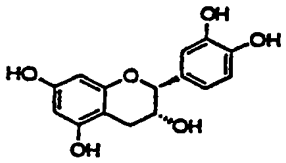
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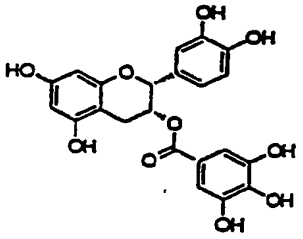
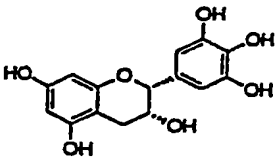
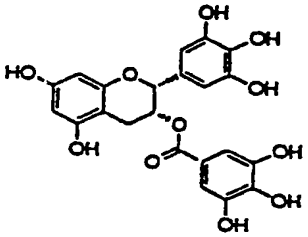
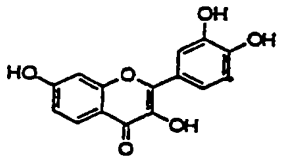

EPICATECHIN GALLATE


EPIGALLOCATECHIN


EPIGALLOCATECHIN GALLATE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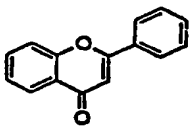
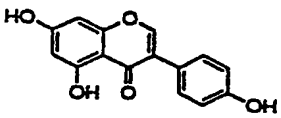
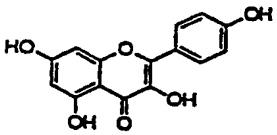

FLAVONE


GENISTEIN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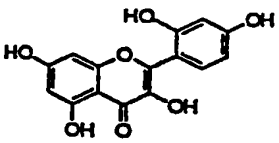
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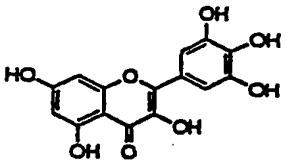
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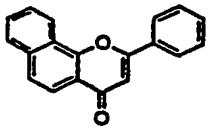
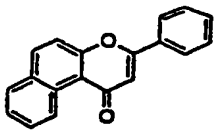
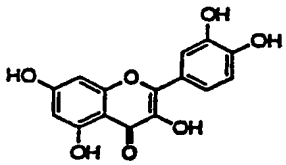
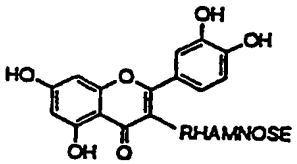

α-NAPTHOFLAVONE


β-NAPTHOFLAVONE


QUERCITIN



RUTIN

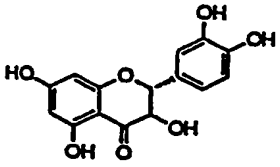
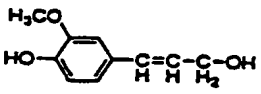
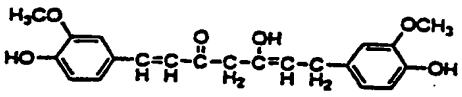

TAXIFOLIN

FIGURE 3 - FERULIC ACID DERIVATIVES

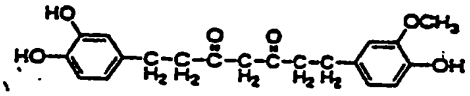
CAPSAICIN



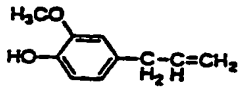
CONIFEROL



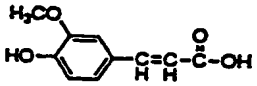
CURCUMIN



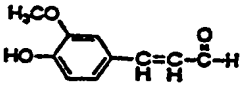
DEMETHOXYTETRAHYDROCURCUMIN



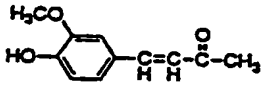
EUGENOL



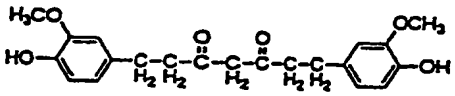
FERULIC ACID



4-HYDROXY-3-METHOXY-CINNAMALDEHYDE

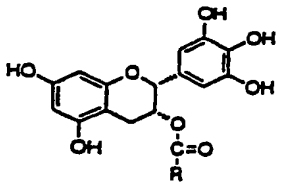


4-(4-HYDROXY-3-METHOXYPHENOL)-3-BUTEN-2-ONE



TETRAHYDROCURCUMIN
HZIV 81-2

FIGURE 5 - EPIGALLOCATECHIN DERIVATIVES



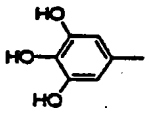
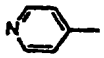
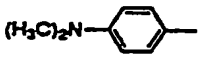
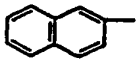
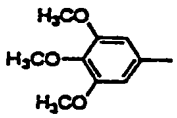
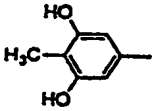
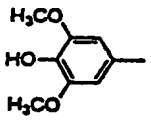
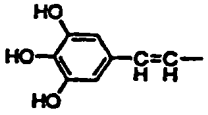
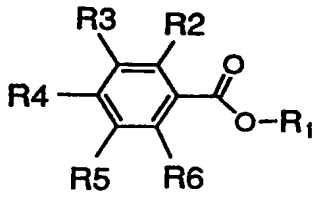
COMPOUND	R
EGCG	
EGC	H
HZIV 109	OH
HZIV 145	CH_3
HZIV 169	$CH_3(CH_2)_4$
HZIV 166	$CH_3(CH_2)_6$
HZIV 168	 (C_9H_{17})
HZIV 165	$CH_3(CH_2)_{12}$
HZIV 160	 $(C_{13}H_{25})$
HZIV 148	$CH_3(CH_2)_{16}$
HZIV 142	 $(C_{17}H_{29})$
HZIV 144	 (CYCLOHEXYL)
HZIV 74	
HZIV 107	
HZIV 92	
HZIV 120	
HZIV 63	
HZIV 68	
HZIV 75	
HZIV 134	

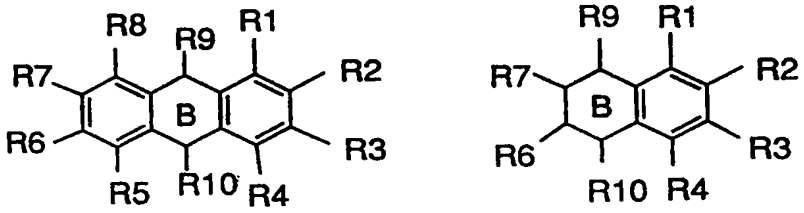
FIGURE 7 - GALLATES



R1: an alkyl chain with 2 to 20 atoms from the group consisting of carbon, oxygen, sulfur, and nitrogen, without or with one to four double bonds and additional hydrogen. These atoms can be in a straight chain or branched form, or in the form of aromatic ring structures, which may have substitution of one to three carbon alkyl or halogenated alkyl, nitro, amino, methylated amino, carboxyl, hydroxy groups or halogen atoms.

R2-5: an alkyl chain with 1 to 12 atoms from the group consisting of carbon, oxygen, sulfur, hydrogen and nitrogen, without or with hydroxy groups. These atoms can be in a straight chain or branched form, which may have substitution of one to three carbon alkyl or halogenated alkyl, nitro, amino, methylated amino, carboxyl groups and hydrogen or halogen atoms.

FIGURE 9 - QUINONES AND CATECHOLS



R1 - R8 can be 1 to 6 atoms that may consist of carbon, nitrogen, oxygen, and sulfur, and additional hydrogen or halogen atoms. They can be in the form of alkyl or halogenated alkyl, methoxy, nitro, hydroxy or amino groups. R9 and R10 can be hydroxy groups or in the form of quinones. Ring B can be in a saturated, aromatic or quinine structures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/23041

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

cas online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,5,605,929 A (LIAO ET AL.) 25 February 1997, column 2 lines 21-32 and 47-56, figures 22 and 23, claims 1-8.	1-10
X	Wang, Z.Y. et al, "Interaction of epicathechins derived from green tea with rat hepatic cytochrome p-450" Chemical Abstracts 108:160935, 1988, see entire abstract.	1-7, 9, 10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

05 JANUARY 1999

Date of mailing of the international search report

03 FEB 1999

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Form PCT/ISA/210 (second sheet)(July 1992)★